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Effect of Probiotics *Bacillus* and Yeast on the Growth Potentials of penaeid prawns *Penaeus monodon* and *Penaeus indicus*

Naresh S[1], Suneetha Y[2], Kumaraswamy Naidu C[2], Srinivasulu Reddy M[2]

1. Department of Zoology, V.S.U.P.G.Center, Kavali, India
2. Department of Zoology, S.V.University, Tirupati-517502, India

Corresponding Author: Department of Zoology, S.V.University, Tirupati-517502, India

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ABSTRACT

Feeding trail experiments were conducted with penaeid prawns *Penaeus monodon* and *Penaeus indicus* after fed with four different types of diets. The Probiotic Diet–I (Commercial diet with *Bacillus* sp.), Probiotic Diet-II (Commercial Diet with *Bacillus* sp. and Yeast sp.) and Probiotic Diet-III (Commercial diet with Yeast sp.) induced relatively higher rates compared to the Control Diet a commercial Diet. *Bacillus* and Yeasts, the probiotics selected in the present study offers a promising alternative to the Antibiotics for sustainable prawn culture activity and hence are recommended for use in prawn culture activity.

Keywords: Aquaculture, Probiotics, *Penaeus monodon*, *Penaeus indicus*

1. INTRODUCTION

Probiotics are live non-pathogenic microorganisms that provide colonization resistance to the pathogenic microbes and thus are effective in prevention and treatment of some diseases. Fuller et al. (1989) defined probiotics as live Microbial feed supplements which beneficially affect the host by improving its intestinal Microbial balance. Probiotics, Lactic acid Bacteria and *Bacillus* sp. as "Bio-friendly agents" can be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms (Gatesoupe, 1999, 2007; Ninawe & Selvin, 2009; Cruz et al., 2012). In recent times, the probiotics which are environmental friendly sustainable approach are used increasingly in penaeid prawn culture activities for reducing undesirable problems, prevention of disease and growth encouragement (Gatesoupe, 1999; Wang, 2007). Probiotics are Microbial supplements to displace pathogens by competitive processes is being used in Aquaculture activity as a better remedy

than administering antibiotics and is now gaining acceptance for the control of pathogens in Aquaculture (Irianto & Austin, 2002). Penaeid prawn culture activity i.e., culture of *P.monodon*, *P.indicus* and *P.vennamei* play an important role in the economy of India, due to great importance in earning of foreign exchange and also to meet up protein demands. At present one of the major problems in the prawn culture is the Microbial diseases caused by Self-pond pollution (Nimrat et al., 2008). Probiotic bacteria improve the health of prawns by controlling pathogens and improving water quality by modifying the microbial community composition of water (Nimrat et al., 2008). The main probiotic Bacteria documented in the prawn grow-out are *Bacillus sp.* strains (Moriarty, 1996; 1998; Thompson et al.,1999) such as *Bacillus subtilis* or Gram–negative Facultative anaerobic bacteria strains, Gram -negative Facultative anaerobic bacteria such as *LactoBacillus* sp.*Roseobacter* sp.*Carnobacterium* sp.*Pseudomonas* sp. especially *Bacillus* (Balcazar et al., 2007). Several Reviews (Cruz et al., 2012; Ninewe & Selvin, 2009; Gatesoupe, 1999) detail the various developments made in the use of probiotics in Aqua cultured species, including penaeid prawns.

Bacillus sp. have possessed the ability of Adhesion, Production of Bacteriocins and providing immunostimulation and more over they have maintained in the spore form that lead to be prolonged self-life (Barbosa et al., 2005). For Yeasts they were used as probiotics based on immunostimulation ability, production of inhibitory compounds and providing protein. Based on the previous research probiotics it is suggested that the use of probiotics *Bacillus* or Yeast, either individually or in combination in Aquaculture has tremendous scope and the study on application of probiotics in aquaculture has a glorious future. The present study therefore has been conducted with the objective of supplementing probiotics in the diet of penaeidprawns *P.monodon* and *P.indicus* and assessing their growth performance and suitability of the *Bacillus* and Yeast by culture activity.

2. MATERIALS AND METHODS

Penaeid prawns *Penaeus monodon* (2.85±0.23G) and *Penaeus indicus*(2.12±0.21G) were selected from the local Aquaculture ponds and were stocked in large Cement tanks (2×1.5×0.75M). The Cement tanks were provided soil collected from Aqua ponds in order to provide earthen pond environment. The Cement tanks were filled with water drawn from nearby Aqua forms. The Hydro biological parameters including Salinity 15±1ppt, Temperature 28±1^{o}C, pH 8.2±0.1, Dissolved oxygen (DO) 6.0±0.2ppm were maintained constantly throughout the experimentation, All the Experimental Tanks were continuously aerated with the help of Air compressors.20% water exchange was done every day in the night after feeding was over. The Experimental Tanks were kept in 12:12 L: D cycle to nullify the effects in any. In the present investigation Twelve tanks were selected allocating Three each to four experimental conditions. Each tank was stocked with 100 numbers of prawns i.e. either *P.monodon* or *P.indicus*, thereby 300 individual prawns were selected to conduct the experiments. The Four groups of Animals kept in different conditions of present investigation are; A. Control Group of Animals fed with commercial diet, B. Probiotic- I: Commercial diet pellets with freeze-dried *Bacillus* probiotics, C. Probiotic- II: Commercial diet with combination of freeze-dried *Bacillus* probiotics and Freeze-dried yeast probiotics and D. Probiotic-III: Commercial diet with freeze-dried yeasts, all the above Formulated Diets are stored in sterile glass bottles and are used in the present experimentation.

The Bacterial probiotics including five strains of *Bacillus* and Two strains of yeasts were used in the present investigation for incorporation in the formulation of Diets. Each strain of *Bacillus* was separately inoculated into a 1 lit flask containing 250ml of Trypticase Soy Broth (TSB) and then flasks were vigorously shaken at kept at 30°C for 24h. In the case of Yeast preparation, a loop full of each yeast species was separately distributed into 1lit flask containing 250ml of yeast Peptone Dextrose broth and kept and 25°Cfor 24h.Cells of both Bacteria and Yeast probiotics were harvested by centrifugation at 8000rpm at 4°C for 5minutes and washed gently with Phosphate Buffer solution. Then the cell suspensions were adjusted to 1.5AU i.e., approximately 10^{10} CFU/ml using Spectrophotometer at 580 nm. The cell suspension was freshly prepared for further production as freeze-dried and is subsequently used in the present experimentation.

Prawns samples were collected than each group on 50th and 100th day of experiment. Microbiological determination studies were conducted on 0, 50 and 100th day of experiment. All experimental groups of prawns were fed daily *adlibitum* at the of 5-6% body weight with the above mentioned Four different types of probiotic feeds at 6.00pm for 2-3 h and left over feed and faecal materials were siphoned out from the Experimental tanks. The prawns were dissected using sterilized surgical scissors and the Gut portion was removed for microbial enumeration and identification. The Gut samples were homogenized using 0.85% NaCl solution and serially diluted for viable plate counts. The diluted sample of 100µlit was pipetted out for Agar spread plating. The Total Heterotrophic Bacterial and *Bacillus* counts were determined by using plate count Agar and the inoculated plates were incubated at 30°C for 48 h (Shariff etal., 2001;Nimrat etal., 2008). Yeast probiotics were enumerated using Yeast Peptone Dextrose Agar at 25°Cfor 48h.Then, all the colonies were counted and expressed as CFU/g. The representative colonies were identified based on Morphological and Biochemical characteristics (Krieg & Holt, 1984).

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The duration of the experiment was 100days.During and at the end of the experiment Growth parameters, Total biomass produced, percent survival values and Food conversion ratio values were monitored and recorded. The data obtained in the present investigation was subjected to Statistical Analysis through MS Excel of 2010 version.

3. RESULTS AND DISCUSSION

After the feeding trail experiments at the end of 100 days of experimentation with juvenile penaeid prawns *Penaeus monodon* and *Penaeus indicus* after fed with one Control commercially important diet and Probiotic Diets I, II &III were monitored and recorded. The parameters including Growth potentials, Population counts of Bacteria, Yeast, estimation of Total biomass, Percent survival and Food Conversion Ratio were recorded at the start of the experiment i.e.,0, 50 and 100 days of experimentation. The trends obtained for above parameters in the case of both the penaeid prawns are almost following the same trend. The *Bacillus* population recorded at 0 days of experiment in the Control group is 3.88 ± 0.23 ×10^4CFU/g for *P.monodon* and for *P.indicus* in 3.29±0.34×10^4CFU/g. The *Bacillus* population was increased from 0 day of experiment (DOE) from Control to 50 days of experiment 7.39± 0.93×10^4 CFU/g and subsequently decreased to 4.25±0.39×10^4 at 100 days of experiment period (Table 1). Similarly *P.indicus* experimentation also recorded 3.29±0.34×10^4 at 0 days of culture in Control followed by 6.88± 0.75×10^4 CFU/g at 50 DOEand 3.94±0.43×10^4 CFU/g at 100 DOE. When the prawns were fed with Probiotic Diets-I, II and III showed a significant increment in the population of *Bacillus* in all the DOE i.e., 0, 50 and 100 days. Similarly the occurrence of yeast populations in the gut of prawns at 0, 50 and 100 DOE were presented in Table 2. The parameters associated growth potentials and other related biological parameters in prawns were monitored and presented in Table 3. The growth potentials recorded for *Penaeus monodon* is as follows: in the Control there is a weight gain of 46.53±0.43g, in the case of prawns fed with Probiotic diet- I, II and III as 75.5±0.56 g, 61.35±0.51 g, and 67.44±0.47g, respectively. The total biomass produced for the Control, Probiotic diet-I, PBD-II and PBD-III of *P.monodon* is 13.629, 21.888, 18.098, 19.424kg, respectively. The Percent survival values recorded maximum of 94% with PBD-II, 93% for PBD-I, and 92% for PBD-III and Control. The Food Conversion Ratio values obtained for the Four Diets i.e., maximum recorded with Control is 2.48, followed by 1.62 for PBD-III, 1.49 for PBD-II and 1.27 for PBD-I.

Similarly *P.indicus* also recorded the growth potentials and other parameters as follows. Maximum growth rates were recorded with PBD-I 60.95g, followed by 58.02g, 56.17g and 38.22g, for PBD-II, PBD-III and Controls, respectively. The total biomass produced after feeding with 17.803g with PBD-II, followed by 16.771, 16.446, 11.254 kgs, respectively for PBD-II, PBD-III and Controls. The FCR values recorded to be maximum with 2.85 in the control followed by 2.04, 1.98, 1.82, for PBD-III, PBD-II and PBD-I, respectively. The percent survival values obtained maximum 94% with PBD-I, PBD-III followed by 93% for Control and PBD-II.

The Results obtained for *Bacillus* population in the Guts of Prawns experiment clearly demonstrate that, the *Bacillus* population in the Gut of prawns fed with PBD-I&II was significantly higher compared with Control and PBD-III fed prawns. The results obtained in the present investigation revealed that *Bacillus* population showed a significant increase in the PBD-I fed prawns. The results obtained are in accordance with the results of Rengpipat et al., (1998) in *P.monodon,* who found that *Bacillus* SII population in the tiger shrimp intestine markedly increased when probiotics were added in tiger shrimp diet for 100 days. *Bacillus* SII population in guts of Black Tiger shrimp in probiotic-treated groups increased more than 50% compared to those in the control (Rengpipat et al., 2000). Kesarcodi-Watson et al., (2008) reported that *Bacillus* are accepted as putative probiotics capable of competing for adhesion sites and nutrients through production of inhibitory substances and active proliferating in digestive tract of aquatic animals. So the presence of the *Bacillus* probiotic significantly improved shrimp survival in most treatments. Because administration of the probiotic significantly changed the proportion of *Bacillus* bacteria in the gut flora, the increased survival by prawns may be due to exclusion of other bacteria (especially harmful bacteria) by the probiont. In *P.monodon*, *Bacillus*, used as a probiotic, was able to colonize both the culture water and the shrimp digestive tract; the *Bacillus* also was able to replace *Vibrio* sp. in the gut of the shrimp, thereby increasing shrimp survival (Rengpipat et al., 1998a). *Bacillus* bacteria are able to out-compete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics (Moriarty, 1998; Verchucre et al., 2000). *Bacillus* administration also has been shown to increase shrimp survival by enhancing resistance to pathogens by activity both cellular and humoral immune defences in shrimp (Rengpipat et al., 2000). Administration of the *Bacillus* bacteria to shrimp is known to increase the production of relatively higher amount of Lipases, Proteases and Amylases from the shrimp digestive tract. Because gram-positive bacteria, particularly member of the genus *Bacillus*, do secrete a wide range of exozymes (Moriarty, 1996; 1998). The increased levels of digestive enzymes due to probiotic treatments may have fed to enhancement digestion and increased absorption of food, which in turn contributed to the improved survival and also growth rates in the prawns. Similar kind of situation also may prevail which lead to better growth rates in probiotic treated prawns compared to Control group of prawns. The FCR values and growth rates obtained in the present investigation also clearly demonstrate that *Bacillus* addition in the food, capable of inducing significant induction of growth potentials and also survival rates.

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In recent years several Researchers reported the application of Yeast as probiotics and their role in culture activity of prawns or shrimps also in marine culture activity. The yeast concentrations in the intestine of sea bass *Dicentrachus labrax* was increased significantly consequent upon the administration of *Debaryomyceshansenii*HF1 as probiotics into sea bass diets. It is indicated that yeasts can adhere and survive in the intestine of aquatic biota. Significant enhancement of percentage of weight gain in PBD-I, II&III in the present study suggests that *Bacillus* and yeasts are authorized as probiotics and are more appropriate for cultivation of both penaeid prawns *P.monodon* and *P.indicus*. Our results also gains support from the literature that the phenomenon of inclusion of *Bacillus* and Yeasts has been attributed from biosynthesis of extracellular enzymes responsible for digestion and assimilation processes in prawn gut or intestine, such as Proteases, Carbohydrases and Lipases as well as providing necessary growth factors (Arellano & Olmos, 2002; Ochoa & Olmos, 2006). Generally, yeast can secrete polyamines, the ubiquitously natural substances, that play a integral role in proliferation and differentiation of cell, advocate the growth and metabolism of aquatic animal (Stefan et al., 2009) as well as adhesive capacity to intestinal mucus that are in turn explain increase in number of yeast in prawn intestine or gut.

From the results obtained in the investigation it concluded that due to incorporation of *Bacillus* and Yeasts in the Commercial diets, the growth potentials of penaeid prawns *P.monodon* and *P.indicus* were significantly enhanced and were demonstrated through percentage of weight gain and also FCR values. *Bacillus* and Yeast, the Probiotics selected in the present investigation offers a promising alternative to the antibiotics for prawn culture activity may be established. Further research is still needed to detect the mode of action of Probiotic on *P. monodon* and *P.indicus* and its effect on immune response and stress management there by establishment of highest growth potentials.

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REFERENCES

1. Arellano C, Olmos S.J. Thermo stable a-1, 4-and a-1-6-glucosidase enzymes from Bacillus sp. isolated from a marine environment. *World. J. Microbial. Biotech*, 1990, 18, 791-795
2. Balcazer JL, Rojas-Luna T, Cunningham DP. Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp Lito penaeus vannamei following immersion challenge with Vibrio parahaemolyticus. *Inv. Pathol*, 2007, 96, 147-150
3. Barbosa TM, Serra CR., La Ragione RM, Wood ward MJ, Henrique's AO. Screening for Bacillus isolates in the broiler gastrointestinal tract. *Appl.Environ.Microbiol*, 2005, 71, 968-978
4. Cruz PM, Ana LI, Oscar AM, Hugo CR. Use of probiotics in aquaculture. International Scholarly Research Network. *ISRN Microbiology*, 2012, 916845, 1–14.
5. Fuller R, Gate soupe FJ. Probiotics in Man and Animals. The use of Probiotics in Aquaculture. *J. Appl. Bacterial*, 1989, 66, 365-378
6. Gate soupe FJ. Live Years in the gut; National occurrence; dietary introduction and their effects on fish health and development. *Aquaculture*, 2007, 267, 20-30
7. Irianto A, Austin B. Probiotics in Aquaculture. *Journal of Fish. Diseases*, 2002, 25, 1-10
8. Kesarcodi Watson A, Kaspar H, Lategan MJ, Gibson L. The need, Principles and Mechanisms of action and screening process. *Probiotics Aquaculture*, 2008, 274, 1-14
9. Krieg NR, Holt JG, Williams & Wilkins Co Baltimore. Bergey's Manual of Systematic Bacteriology, vol. 1, 1984, 161-172
10. Moriarty DJW. Control of Luminous Vibrio species in penaeid Aquaculture ponds. *Aquaculture*, 1998, 164, 351-358
11. Moriarty DJW. A key ingredient for sustainable. Aquaculture Info fish. Int Microbial Biotechnology, 1998, 4, 29-33
12. Nimrat S, Suksawat S, Maleeweach P, Chai V.V. Effect of different shrimp pond bottom soil treatments on the change of physical characteristics and pathogenic bacteria in pond bottom soil. *Aquaculture*, 2008, 285, 123-129
13. Nimrat S, Suksawat S, Maleeweach P.Vuthiphandchai V. Effect of different shrimp pond bottom soil treatments on the change of physical characteristics and pathogenic bacteria on pond bottom soil. *Aquaculture*, 2008, 285, 123-129
14. Ninawe AS, Selvin S. Probiotics in Shrimp Aquaculture: Avenues and challenges. *Critical Reviews in Microbial*, 2009, 35(1), 43-66
15. Ochoa-Solano JL, Olmos-Soto J. The functional properly of Bacillus for shrimp feeds. *Food Microbiology*, 2006, 23, 519-525
16. Rengpipat S, Rukpratanporn S, Piyatirattivorakul S, Menasveta P. Immunity enhancement on black tiger shrimp Penaeus monodon by a probiont bacterium Bacillus SII. Aquaculture, 2002, 191, 271-288
17. Rengpipat S, Phianphak W,Piyatiratitivorakul S, Menasveta P. Effects of a probiotic bacterium on black tiger shrimp Penaeus monodon survival and growth. Aquaculture, 1998a, 167, 301-313
18. Sharif M, Yusoff FM, Devaraja TN, Rao SPS. The effectiveness of a commercial Microbial product in poorly prepared tiger shrimp Penaeus monodon (Fabricus) ponds. *Aqua*. *Res*, 2001, 32, 181-187
19. Stefan D, Staykor Y, Rumyana M, Georgi B. Microbial ecology of the gastrointestinal tract of fish and the potential application of pro biotic and pre biotic in fin fish aquaculture. *Int. Aqua. Res*, 2009, 1, 1-29
20. Thompson FL, Abreu PC, Cavalli R. The use of Microorganisms as food source for Penaeuspaulensis larvae. *Aquaculture*, 1999, 174, 139-153
21. Wang YB. Effect of Probiotics on growth performance and digestive enzyme activities of the shrimp Penaeus svannamei. *Aquaculture*, 2009, 269, 259-264

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Table 1

Occurrence of Bacillus Population on the gut penaeid Prawn *Penaeus monodon* and *Penaeus indicus.*

Days of culture	Bacillus population population(CFU/g) on *P.monodon*			
	Control	Probiotic-II	Probiotic-II	Probiotic-III
0	$3.88\pm0.23\times10^{4}$	$7.23\pm0.54\times10^{5}$	$7.38\pm0.61\times10^{5}$	$4.25\pm0.41\times10^{4}$
50	$7.39\pm0.93\times10^{4}$	$2.37\pm0.39\times10^{9}$	$2.21\pm0.43\times10^{9}$	$8.33\pm0.67\times10^{4}$
100	$4.25\pm0.39\times10^{4}$	$3.75\pm0.45\times10^{9}$	3.43±0.59×109	5.05±0.75×104

The values are statistically significant from control at P< 0.001

Table 2

Occurrence of yeast population in the gut of penaeid prawn *Penaeus monodon* and *Penaeus indicus.*

Days of culture	Yeast population (CFU/g) on *P. indicus*			
	Control	Probiotic-II	Probiotic-II	Probiotic-III
0	$3.29\pm0.34\times10^{4}$	$6.89\pm0.65\times10^{5}$	$7.23\pm0.65\times10^{5}$	$4.14\pm0.38\times10^{4}$
50	$6.88\pm0.75\times10^{4}$	$2.52\pm0.39\times10^{8}$	$2.18\pm0.39\times10^{8}$	$7.58\pm0.72\times10^{4}$
100	$3.94\pm0.43\times10^{4}$	$3.69\pm0.72\times10^{8}$	$3.13\pm0.75\times10^{8}$	$4.93\pm0.62\times10^{4}$

The values are statistically significant from control at P< 0.001

Table 3a

Growth Parameters in *P. monodon* after fed with Control and Different Probiotic Diets

	P.monodon						
	IW	FW	WG	%WG	Total Biomass(kg)	FCR	% Survival
Control	2.85±0.23	49.38±0.45	46.53±0.43	1633*	13.629	2.48	92
Probiotic-I	2.91±0.28	78.45±0.58	75.54±0.56	2596*	21.888	1.27	93
Probiotic-II	2.83±0.25	64.18±0.54	61.35±0.51	2168*	18.098	1.49	94
Probiotic-III	2.94±0.27	70.38±0.49	67.44±0.47	2294	19.424	1.62	92

The values are statistically significant from control at P<0.001

Table 3b

Growth Parameters in *P. indicus* after fed with Control and Different Probiotic Diets

	P. indicus						
	IW	FW	WG	%WG	Total Biomass(kg)	FCR	% Survival
Control	2.12±0.21	40.34±0.38	38.22±0.39	1802*	11.254	2.85	93
Probiotic-I	2.18±0.24	36.13±0.39	60.95±0.37	2796*	17.803	1.82	94
Probiotic-II	2.09±0.23	60.11±0.33	58.02±0.31	2776*	16.771	1.98	93
Probiotic-III	2.15±0.23	58.32±0.35	56.17±0.32	2613*	16.446	2.04	94

The values are statistically significant from control at P<0.001

IW: Initial weight, FW: Final weight, WG: weight gain, %WG: percent weight gain

TB: Total Biomass, % survival, FCR: Food Conversion Ratio

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